UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

September 14, 2009

MEMORANDUM

Subject:	Name of Pesticide Product:	EVERCIDE MICROEMULSION PET PUMP SPRAY 2649
	EPA Reg. No. /File Symbol:	1021-1726
	DP Barcode:	D367106
	Decision No.:	415029
	Action Code:	R340
	PC Codes:	057001 (MGK 264); 069001 (Pyrethrins); 109701 (Permethrin); & 129032 (Pyriproxyfen)

From: Byron T. Backus, Ph.D., Toxicologist
Technical Review Branch
Registration Division (7505P)

Byron T. Backus
9-14-2009
M Hawkins - Toxicology Team Leader

To: Kevin Sweeney/Richard Gebken, RM 13
Insecticide Branch
Registration Division (7505P)

Registrant: MCLAUGHLIN GORMLEY KING CO.

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
069001 Pyrethrins	0.112%
109701 Permethrin (CAS #52645-53-1)	0.100%
Related compounds	0.009%
057001 N-Octyl bicycloheptene dicarboximide	0.500%
129032 2-[1-Methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine	0.125%
Other Ingredient(s):	99.154%
TOTAL	100.000%

ACTION REQUESTED: The Risk Manager requests:

"...see instructions for 1021-1725. These studies [MRID 47775101, a companion animal safety study with puppies, and MRID 47775102, a companion animal safety study with adult dogs] are being used to support both products."

BACKGROUND:

The material received for review under DP Barcodes 367098 and 367106 includes two companion animal safety studies (MRID 47775101, a companion animal safety study with approximately 12-week old beagle puppies; and MRID 47775102, a companion animal safety study with "adult" beagles).

COMMENTS AND RECOMMENDATIONS:

1. TRB conducted the primary review of the companion animal safety studies in MRIDs 47775101 and 47775102.

2. The following is the executive summary from the review of the study in MRID 47775101:

In a companion animal safety study (MRID 47775101), six 12-week old beagle puppies/sex/group received topically a spray of either placebo or formulations containing various concentrations of several active ingredients. Although the formulations did not include Nylar, the study in MRID 42178309 indicates a low mammalian toxicity for this active, and can be cited. Group I puppies were sprayed with X-6415-08, a placebo formulation at a mean dosage of 1.7 g/kg. Group II puppies were sprayed with X-6422-09, a 1x formulation [with 1.07% N-Octyl bicycloheptene dicarboximide; 0.529% Piperonyl butoxide; 0.307% Pyrethrins; 0.210% Bifethrin; 0.213% Permethrin; and 0.102% ETOC] at a mean dosage rate of 1.9 g/kg. Group III puppies were sprayed with X-6423-09 [3.19% N-Octyl bicycloheptene dicarboximide; 1.53% Piperonyl butoxide; 0.94% Pyrethrins; 0.604% Bifethrin; 0.614% Permethrin; and 0.308% ETOC] at a mean dosage rate of 1.5 g/kg. Group IV puppies were sprayed with X-6414-08 [5.01% N-Octyl bicycloheptene dicarboximide; 2.45% Piperonyl butoxide; 1.64% Pyrethrins; 0.996% Bifethrin; 1.02% Permethrin; and 0.532% ETOC] at a mean dosage rate of 1.6 g/kg. Following treatment, individual puppies were observed at 1-hour intervals for 6 hours. They were then observed (with a.m. and p.m. observations) for 14 days.

During the pretest period, very slight to moderate ocular discharge was observed in 21 puppies, and nictitans gland prolapse (cherry eye) was observed in two. On Day -8 puppy 4263-M (later assigned to Group II) was slightly dehydrated and was found on fecal examination to have Giardia and coccidiosis. This puppy was treated with Metronitazole for 5 days and with FortiFlora for 7 days. All other puppies were treated with Panacur. One puppy's ears were treated for 7 days with Malacetic Otic. The eyes of 15 puppies were treated for 7-8 days with Vetropolycin.

During the immediate post-dose observations soft feces ("very slight" to "slight") were observed in one Group II male, one Group III male, and one Group IV male; an additional Group IV male, #4282, showed moderate diarrhea at 4 hours post-dose and also showed slight diarrhea at the PM observation on Day 0. Diarrhea was also observed in one Group I male on Day 5 (AM

observation). Other signs noted in the period from Day 1 to 14 included ocular discharge in two Group IV males at the AM and PM observations on Day 5 and "cherry eye" (for both eyes) in one Group IV male from the AM observation on Day 8 through the PM observation on Day 14. An additional Group IV male (#4283-M) had cherry eye from Day 1 through Day 14, and a Group II female (#4261-F) had cherry eye from pretest through Day 14.

Overall, there is no indication that exposure to the formulations (which included, for the 3x and 5x groups, exaggerated amounts of the active ingredients) resulted in any significant adverse toxicological effects. However, there are several deficiencies that must be corrected before this study can be classified as acceptable to support this product's use on 12-week old puppies. These are the following:

1) The composition of the placebo formulation (X-6415-08) should be provided, as well as information regarding the percentage(s) of inert(s) present in the other test formulations. In addition, a clarification is needed as on p. 6 of MRID 47775101 it is stated that: "On Day 0, Group I animals were treated with X-6415-08, a 5X dose of the placebo and served as controls." The mean dosage rate at which X-6415-08 was applied in Group I puppies was 1.7 g/kg, essentially the same dosage rate (1.9 g/kg) at which X-6422-09 was applied to Group II puppies, so it appears that the X-6415-08 was applied at a 1X application rate.

2) The dates of birth for the individual puppies should be reported to establish that they were in fact no more than 12 weeks old at treatment.

3) The product label should be revised to give dosage rates in terms of duration of spray (in seconds) based on a puppy's weight.

This companion animal safety study (OPPTS 870.7200) in 12-week old puppies is currently classified as unacceptable. It can be upgraded to acceptable provided the deficiencies indicated above are satisfied.

3) The following is the executive summary from the review of the study in MRID 47775102:

In a companion animal safety study (MRID 47775102), six adult (ages not specified) dogs/sex/group were topically sprayed on Days 0 and 7 with either a placebo (Group I) or a formulation (X-6414-08, containing 5.01% N-Octyl bicycloheptene dicarboximide; 2.45% Piperonyl butoxide; 1.64% Pyrethrins; 0.996% Bifethrin; 1.02% Permethrin; and 0.532% ETOC. Although the formulations did not include Nylar, the study in MRID 42178309 indicates a low mammalian toxicity for this active, and can be cited. The mean dosage rate for dogs sprayed with the X-6414-08 for both occasions was ~0.95 g/kg. Following treatment, individual dogs were observed at 1-hour intervals for 6 hours. They were observed (with a.m. and p.m. observations) for 21 days after the Day 0 application.

During the pretest period one Group II male favored one back leg on Days -16 through -12, and another Group II male had a growth/cyst between toes of the right foot, diagnosed as an interdigital abscess which was treated with full recovery by Day 1. One Group I male (4160-M) was not eating at the p.m. observations on Days -2 and -1; from the food consumption data this dog ate nothing on Days -11, -8, -4, -3, and -1, and consumed only 5 g of food on Day -2 and

6.2 g on Day 0. 4160-M was dehydrated on Day 1 and was treated [presumably intravenously] with 120 mL of sterile saline and was given antibiotics and fed Pedigree canned food on three occasions. This animal was also given an extra 25 g of regular food on at least four occasions (Days 14, 15, 16 and 20). From the food consumption data, several other dogs (Group II male 4161 on Days -11 and -12, Group II male 4163 on Day -11, Group I female 4180 on Day -6, Group II female 4177 on Day -5) had days when they did not consume any food.

From Day -20 to 0 Group I dogs went from a mean body weight of 8.3 to 7.2 kg, and Group II dogs went from a mean body weight of 8.3 to 7.7 kg. No explanation is provided as to why these weight losses occurred.

During the immediate post-dose observations on Day 0 one Group II male had very slight lacrimation of the left eye at 6 hours and one Group II female had very slight lacrimation in one or both eyes at 1 hour through 6 hours; this same female also showed very slight lacrimation in both eyes at the pm observation on Day 0, the pm observation on Day 1 and the am observation on Day 2. Nothing was observed in any dog following treatment on Day 7.

One Group I male (4166-M) is reported as showing very slight salivation at the am observation on Day 1, one Group I female (4181-F) showed very slight lacrimation at the pm observation on Day 9 and a Group I female appeared thin on Days 20-21.

Four Group II dogs (4163-M, 4164-M, 4177-F and 4178-F) had low (<100 g) food consumption on Day 0, while only two Group I dogs (including 4160-M, which had not been eating well for several days) also had low food consumption. The mean food consumption for Group II females on Day 0 was 133.7 g, while for Group I females it was 225.1 g. Except for one male (4164-M) which consistently ate less than 200 g/day from Day 0 to 7, all Group II dogs consumed more than 200 g of food on Day 7. On Day 8 (the day following the second treatment), four (3 female, 1 male) Group II and two (both males) Group I dogs consumed less than 200 g. The food consumption data suggest (but do not conclusively demonstrate) a temporary decrease in appetite in some dogs, particularly females, following treatment with X-6414-08. However, this is not considered an indication of a serious adverse toxicological response.

Overall, there is no indication that exposure to X-6414-08 (which contained 5x amounts of the active ingredients) at a mean dose of 0.95 g/kg resulted in any significant adverse toxicological effects. However, there are several deficiencies that should be corrected before this study can be classified as acceptable to support this product's use on adult dogs. These are the following:

1) The composition of the placebo formulation (X-6415-08) should be provided, as well as information regarding the percentage(s) of inert(s) present in X-6414-08.

2) The approximate ages of the individual dogs should be reported.

3) There should be an explanation provided as to why a number of dogs had weight losses in the period from Day -2- to 0. During this period Group I dogs went from a mean body weight of 8.3 to 7.2 kg, and Group II dogs went from a mean body weight of 8.3 to 7.7 kg. An explanation should also be provided as to why Group I male 4160-M was included in this study, as this animal was eating very little in the period from Day -5

through Day 0 (according to the food consumption data this dog ate a total of 34.9 grams of food during this period), and was obviously not in good physical condition as it was dehydrated on Day -1.

4) Information should be given as to whether the dogs were fed before or after treatment on Days 0 and 7.

5) The product label should be revised to give dosage rates in terms of duration of spray (in seconds) based on a dog's weight.

This companion animal safety study (OPPTS 870.7200) in adult dogs is currently classified as unacceptable. It can be upgraded to acceptable provided the deficiencies indicated above are satisfied.

EPA Reviewer: Byron Backus, Ph.D. **Signature:** Byron T. Backus
Technical Review Branch, Registration Division (7505P) **Date:** 9-14-2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion animal safety study- beagle puppies; [OPPTS 870.7200]

PC CODES: 057001, 067501, 069001, 128825, 109701, 128722 **DP BARCODE:** 367106

TEST MATERIALS (PURITY): X-6422-09: N-Octyl bicycloheptene dicarboximide (1.07%); Piperonyl butoxide (0.529%); Pyrethrins (0.307%); Bifethrin (0.210%); Permethrin (0.213%); ETOC (0.102%) [used for 1x]. N-Octyl bicycloheptene dicarboximide (1.07%); Piperonyl butoxide (0.529%); Pyrethrins (0.307%); Bifethrin (0.210%); Permethrin (0.213%); ETOC (0.102%) [used for 1x]. X-6423-09: N-Octyl bicycloheptene dicarboximide (3.19%); Piperonyl butoxide (1.53%); Pyrethrins (0.940%); Bifethrin (0.604%); Permethrin (0.614%); ETOC (0.308%) [used for 3x]. X-6414-08: N-Octyl bicycloheptene dicarboximide (5.01%); Piperonyl butoxide (2.45%); Pyrethrins (1.64%); Bifethrin (0.996%); Permethrin (1.02%); ETOC (0.532%) [used for 5x].

SYNONYMS: X-6422-09 (CASS 1x Formula); X-6423-09 (CASS 3x Formula); X-6414-08 (CASS 5x Formula).

CITATION: Kuhn, J. (2009) X-6414-08, X-6422-09 and X-6423-09: Companion Animal Safety Study in Puppies: Final Report. Project Number: 12418/08. Unpublished study prepared by Stillmeadow, Inc. 52 p. Unpublished. Feb. 10, 2009.

SPONSOR: McLaughlin Gormley King Company

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 47775101), six 12-week old beagle puppies/sex/group received topically a spray of either placebo or formulations containing various concentrations of several active ingredients. Although the formulations did not include Nylar, the study in MRID 42178309 indicates a low mammalian toxicity for this active, and can be cited. Group I puppies were sprayed with X-6415-08, a placebo formulation at a mean dosage of 1.7 g/kg. Group II puppies were sprayed with X-6422-09, a 1x formulation [with 1.07% N-Octyl bicycloheptene dicarboximide; 0.529% Piperonyl butoxide; 0.307% Pyrethrins; 0.210% Bifethrin; 0.213% Permethrin; and 0.102% ETOC] at a mean dosage rate of 1.9 g/kg. Group III puppies were sprayed with X-6423-09 [3.19% N-Octyl bicycloheptene dicarboximide; 1.53% Piperonyl butoxide; 0.94% Pyrethrins; 0.604% Bifethrin; 0.614% Permethrin; and 0.308% ETOC] at a mean dosage rate of 1.5 g/kg. Group IV puppies were sprayed with X-6414-08 [5.01% N-Octyl bicycloheptene dicarboximide; 2.45% Piperonyl butoxide; 1.64% Pyrethrins; 0.996% Bifethrin; 1.02% Permethrin; and 0.532% ETOC] at a mean dosage rate of 1.6 g/kg. Following treatment, individual puppies were observed at 1-hour intervals for 6 hours. They were then observed (with a.m. and p.m. observations) for 14 days.

During the pretest period, very slight to moderate ocular discharge was observed in 21 puppies,

and nictitans gland prolapse (cherry eye) was observed in two. On Day -8 puppy 4263-M (later assigned to Group II) was slightly dehydrated and was found on fecal examination to have Giardia and coccidiosis. This puppy was treated with Metronitazole for 5 days and with FortiFlora for 7 days. All other puppies were treated with Panacur. One puppy's ears were treated for 7 days with Malacetic Otic. The eyes of 15 puppies were treated for 7-8 days with Vetropolycin.

During the immediate post-dose observations soft feces ("very slight" to "slight") were observed in one Group II male, one Group III male, and one Group IV male; an additional Group IV male, #4282, showed moderate diarrhea at 4 hours post-dose and also showed slight diarrhea at the PM observation on Day 0. Diarrhea was also observed in one Group I male on Day 5 (AM observation). Other signs noted in the period from Day 1 to 14 included ocular discharge in two Group IV males at the AM and PM observations on Day 5 and "cherry eye" (for both eyes) in one Group IV male from the AM observation on Day 8 through the PM observation on Day 14. An additional Group IV male (#4283-M) had cherry eye from Day 1 through Day 14, and a Group II female (#4261-F) had cherry eye from pretest through Day 14.

Overall, there is no indication that exposure to the formulations (which included, for the 3x and 5x groups, exaggerated amounts of the active ingredients) resulted in any significant adverse toxicological effects. However, there are several deficiencies that must be corrected before this study can be classified as acceptable to support this product's use on 12-week old puppies. These are the following:

1) The composition of the placebo formulation (X-6415-08) should be provided, as well as information regarding the percentage(s) of inert(s) present in the other test formulations. In addition, a clarification is needed as on p. 6 of MRID 47775101 it is stated that: "On Day 0, Group I animals were treated with X-6415-08, a 5X dose of the placebo and served as controls." The mean dosage rate at which X-6415-08 was applied in Group I puppies was 1.7 g/kg, essentially the same dosage rate (1.9 g/kg) at which X-6422-09 was applied to Group II puppies, so it appears that the X-6415-08 was applied at a 1X application rate.

2) The dates of birth for the individual puppies should be reported to establish that they were in fact no more than 12 weeks old at treatment.

3) The product label should be revised to give dosage rates in terms of duration of spray (in seconds) based on a puppy's weight.

This companion animal safety study (OPPTS 870.7200) in 12-week old puppies is currently classified as unacceptable. It can be upgraded to acceptable provided the deficiencies indicated above are satisfied.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided, with disclaimers (p. 3 of MRID 47775101) that stability information was not provided to the testing facility, and that it is not known if the provided analysis for characterization and/or stability was conducted according to GLP standards.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Materials:** X-6422-09 (CASS 1x Formula); X-6423-09 (CASS 3x Formula); X-6414-08 (CASS 5x Formula)

Description:	Milky white liquids
Batch #:	
Purity:	N-Octyl bicycloheptene dicarboximide (1.07%); Piperonyl butoxide (0.529%); Pyrethrins (0.307%); Bifethrin (0.210%); Permethrin (0.213%); ETOC (0.102%) [used for 1x]. N-Octyl bicycloheptene dicarboximide (1.07%); Piperonyl butoxide (0.529%); Pyrethrins (0.307%); Bifethrin (0.210%); Permethrin (0.213%); ETOC (0.102%) [used for 1x]. N-Octyl bicycloheptene dicarboximide (3.19%); Piperonyl butoxide (1.53%); Pyrethrins (0.940%); Bifethrin (0.604%); Permethrin (0.614%); ETOC (0.308%) [used for 3x]. N-Octyl bicycloheptene dicarboximide (5.01%); Piperonyl butoxide (2.45%); Pyrethrins (1.64%); Bifethrin (0.996%); Permethrin (1.02%); ETOC (0.532%) [used for 5x].
Compound Stability:	Not provided for the 1x and 3x formulations; for the 5x and placebo formulations: "September 2010 per provided information."
CAS #:	Not provided

2. **Vehicle control:** A placebo control (X-6415-08) of the formulation without active ingredients was used. No information is provided as to its composition.

3. **Test animals:**

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	Puppies- approximately 12 weeks old at dosing; Day -1 bodyweights: Males: 3.0 - 5.5 kg and Females: 2.9 - 4.5 kg	
Source:	Ridglan Farms, Inc., Mt. Horeb, WI	
Housing:	Individual cages measuring 3' x 4', with wire bottoms.	
Diet:	Canine High Density Diet #5L18 (PMI Nutrition International, Inc.), fed once a day "appropriate amount to meet nutritional requirements." From the maximum food consumption values, each puppy was offered 400 grams of food from Day 0 to Day 5, then was offered 300 grams of food from Day 6 through Day 14.	
Water:	Municipal supply, *ad libitum*	
Environmental conditions:	**Temperature:**	$14\text{-}26^0$ C
	Humidity:	23-91%
	Air changes:	10-12 exchanges/hour
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	~ 2 weeks	

B. STUDY DESIGN:

1. **In life dates:** Start: December 2, 2008 (with dosing on Day 0 or December 3, 2008); End: December 18, 2008.

2. **Animal assignment:** From p. 9 of MRID 47775101: "Using a computer-generated program, animals were randomly assigned to one of four groups, Groups I, II, III or IV, with six males and six females in each group in an effort to equalize the mean body weight and sex."

TABLE 1. Study design[a]				
Test Group	**Number of puppies**	**Dosages (with mean) & Material Applied**	**Day -1 Body wt. in kg (mean)**	**Dosages in g/kg**
I (Placebo)	12 (6 M and 6 F)	M: 5.5-9.0 (6.85) F: 4.4-7.9 (1.31) g/kg X-6415-08 (CASS 5x Placebo)	M: 3.5-4.6 (4.03) F: 3.4-4.4 (3.70)	M: 1.3-2.3 (1.73) F: 1.1-2.3 (1.65)
II (1x)	12 (6 M and 6 F)	M: 5.6-11.7 (7.30) g/kg F: 4.6-7.8 (6.42) g/kg X-6422-09 (CASS 1x)	M: 3.0-5.5 (4.00) F: 2.9-4.4 (3.47)	M: 1.2-3.1 (1.90) F: 1.4-2.5 (1.88)
III (3x)	12 (6 M and 6 F)	M: 4.8-8.0 (6.07) g/kg F: 3.5-7.5 (5.57) g/kg X-6423-09 (CASS 3x)	M: 3.8-4.9 (4.42) F: 2.3-4.5 (3.43)	M: 1.0-1.7 (1.40) F: 1.2-2.1 (1.60)
IV (5x)	12 (6 M and 6 F)	M: 4.9-7.8 (6.23) g/kg F: 4.8-7.3 (6.00) g/kg X-6414-08 (CASS 5x)	M: 3.6-5.0 (4.30) F: 3.2-4.4 (3.58)	M: 1.1-1.7 (1.47) F: 1.4-2.1 (1.70)

[a] Data from p. 14-15 in MRID 47775101

3. **Dose selection rationale:** From p. 7 of MRID 47775101: "This study was conducted to Determine the adequate margin of safety of a test substance following topical application to puppies at one, three and five times the label dose." According to the 870.7200 Companion Animal Safety Guidelines, testing of formulations specifically prepared for a study of this type that contain higher concentrations (3x and 5x) of the active ingredient[s] is not only acceptable, but "preferred."

4. **Preparation and treatment**: On Day 0 the test and control formulations were administered topically. The containers were held upright 6-10 inches from the animal and were moved as the spray was discharged. The spray was "lightly" applied to the entire body with an even coverage until the tips of the hair were moist. The formulation was rubbed into the coat by ruffling the hair to reach the skin. Containers were weighed before and after dosing to determine the amount applied on each puppy. It is stated (p. 9 of MRID 47775101) that Group I puppies were treated on Day 0 with a 5X dose of the placebo, X-6415-08; however, from information on p. 14 Group I puppies were treated with from 1.1-2.3 (mean of 1.7) g/kg, comparable to the dosage of 1.2-3.1 (mean of 1.9) g/kg Group II puppies received.

5. **Statistics**: Individual values and group mean values (with standard deviations) by sex are presented for a number of parameters (food consumption, body weights, serum chemistry, and hematology). However, there is no indication within the report (except possibly the statement on p. 12 of MRID 47775101 that there were no significant changes from Day -1 to Day 1 for hematology and serum chemistry results and therefore no blood was drawn on Day 8) that these values were statistically analyzed.

C. METHODS:

1. **Observations and pre-exposure treatments:** All puppies were observed twice daily from Day -14 through Day 14; in addition, on study day 0 they were also observed hourly for 6 consecutive hours after treatment. Only abnormalities were recorded. During the pretest period, very slight to moderate ocular discharge was observed in 21 puppies, and nictitans

gland prolapse (cherry eye) was observed in two. On Day -8 puppy 4263-M (later assigned to Group II) was slightly dehydrated and was discovered on fecal examination to have Giardia and coccidiosis. This puppy was treated with Metronitazole for 5 days, and with FortiFlora for 7 days. All other puppies were treated with Panacur. One puppy's ears were treated for seven days with Malacetic Otic, and the eyes of 15 puppies were treated for 7-8 days with Vetropolycin. Pre-treatment observations included ocular discharge (described as very slight to moderate) and cherry eye; post-treatment observations included soft feces, diarrhea, ocular discharge, decreased appetite, and cherry eye.

2. **Body weight**: Body weights were recorded on Days -1, 7 and 14.

3. **Food consumption**: Puppies were fed once a day. From p. 10 of MRID 47775101: "Individual food consumption was measured daily through study termination." From the maximum food consumption values, it appears that puppies were offered 400 g/puppy/day from Day 0 through Day 5. Starting on Day 6 and through Day 14 each puppy was apparently only offered 300 g/day.

4. **Hematology & Clinical Chemistry**: Blood was collected from the jugular vein for hematology and clinical chemistry assessments for all puppies on Days -1 and 1. The CHECKED (X) parameters were examined.

   a. **Hematology**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count		Reticulocyte count
	Blood clotting measurements*		Large unstained cells (LUC)
	(Thromboplastin time)		Fibrinogen
X	(Activated Partial Thromboplastin Time)		
X	(Prothrombin time)		

* Recommended for companion animals safety evaluation based on OPPTS 870.7200

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Sodium/Potassium ratio	X	Urea nitrogen*
X	Phosphorus *		Total Cholesterol
X	Potassium* (K)	X	Globulins*
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, eg.,)	X	Total bilirubin *
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Albumin/Globulin ratio
	Lactic acid dehydrogenase (LDH)	X	Direct bilirubin*
X	Alanine aminotransferase (ALT/also SGPT)*		Indirect bilirubin
X	Aspartate aminotransferase (AST/also SGOT)*		BUN/Creatinine ratio
	Gamma glutamyl transferase (GGT)		TCO_2 Bicarbonate
	Glutamate dehydrogenase		Amylase
	Sorbitol dehydrogenase		Lipase

* Recommended for a companion animal safety evaluation based on OPPTS 870.7200

5. **Urinalysis:** No urinalysis was performed. Urinalysis is not specified in the 870.7200 Guidelines.

6. **Sacrifice and Pathology**: None of the puppies died during the course of the study, and a terminal sacrifice is not required for this type of study.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** During the pretest period, very slight to moderate ocular discharge was observed in 21 puppies, and nictitans gland prolapse (cherry eye) was observed in two. On Day -8 puppy 4263-M (later assigned to Group II) was slightly dehydrated and was found on fecal examination to have Giardia and coccidiosis. This puppy was treated with Metronitazole for 5 days and with FortiFlora for 7 days. All other puppies were treated with Panacur. One puppy's ears were treated for 7 days with Malacetic Otic. The eyes of 15 puppies were treated for 7-8 days with Vetropolycin.

   During the immediate post-dose observations soft feces ("very slight" to "slight") were observed in one Group II male, one Group III male, and one Group IV male; an additional Group IV male, #4282, showed moderate diarrhea at 4 hours post-dose and also showed slight diarrhea at the PM observation on Day 0. Diarrhea was also observed in one Group I male on Day 5 (AM observation). Other sign noted in the period from Day 1 to 14 included ocular discharge in two Group IV males at the AM and PM observations on Day 5 and "cherry eye" (for both eyes) in one Group IV male from the AM observation on Day 8 through the PM observation on Day 14. An additional Group IV male (#4283-M) had cherry

eye from Day 1 through Day 14, and a Group II female (#4261-F) had cherry eye from pretest through Day 14.

2. **Cosmetic effects**: No cosmetic effects were observed.

3. **Mortality**: There was no mortality.

B. **BODY WEIGHT AND WEIGHT GAIN**: Select mean body weight data are presented in Table 2. No treatment-related differences in body weight were observed.

TABLE 2. Mean body weight ± S.D. (kg) in puppies treated with X-6414-08, X-6422-09 or X-6423-09[a]

Day	Group I. Placebo	Group II. 1x	Group III. 3x	Group IV. 5x
Males				
-1	4.03 ± 0.40	4.00 ± 0.83	4.42 ± 0.42	4.30 ± 0.47
7	5.00 ± 0.24	4.98 ± 0.93	5.10 ± 0.55	5.07 ± 0.71
14	5.62 ± 0.36	5.58 ± 1.03	5.62 ± 0.51	5.40 ± 1.02
Change	1.58 ± 0.26	1.58 ± 0.54	1.20 ± 0.34	1.10 ± 0.63
Females				
-1	3.70 ± 0.37	3.47 ± 0.54	3.43 ± 0.77	3.58 ± 0.51
7	4.38 ± 0.61	4.22 ± 0.67	4.27 ± 0.75	4.33 ± 0.39
14	4.78 ± 0.66	4.72 ± 0.69	4.60 ± 0.87	4.80 ± 0.36
Change	1.08 ± 0.38	1.25 ± 0.42	1.17 ± 0.36	1.22 ± 0.19

[a]Values calculated from Table I, pages 14 and 15 in MRID 47775101.

C. **FOOD CONSUMPTION:** Select mean food consumption data are presented in Table 3. Although some Group I females, Group III males and females, and Group IV females had reduced mean food consumption values on Day 0, there was no indication of a dose relationship. There was apparently a change in the amount of food offered; prior to Day 6 there were a number of individual daily food consumption values greater than 300 grams (the maximum recorded was 400 g) and from Day 6 the maximum values were 400 grams.

TABLE 3. Mean daily food consumption ± S.D. (g) in puppies treated with X-6414-08, X-6422-09 or X-6423-09[a]				
Day	**Group I (placebo)**	**Group II (1x)**	**Group III (3x)**	**Group IV (5x)**
Males				
0	303.3 ± 148.9	232.3 ± 193.9	56.7 ± 29.0	209.3 ± 149.2
1	338.5 ± 67.7	319.2 ± 71.8	297.5 ± 57.3	332.2 ± 74.9
2	307.9 ± 45.3	297.8 ± 106.2	249.3 ± 51.2	318.9 ± 84.6
3	229.0 ± 38.5	248.9 ± 42.9	242.0 ± 30.0	249.5 ± 85.1
4	178.4 ± 79.4	164.2 ± 65.1	179.6 ± 46.1	141.7 ± 108.4
5	306.4 ± 75.5	264.3 ± 85.1	245.8 ± 18.4	253.5 ± 73.0
6	247.4 ± 49.9	237.2 ± 70.3	198.2 ± 55.9	245.1 ± 60.9
Mean 0-14	271.0 ± 31.4	252.3 ± 58.4	231.9 ± 18.3	261.6 ± 38.9
Females				
0	64.8 ± 14.6	177.8 ± 192.7	79.5 ± 19.3	100.8 ± 75.4
1	299.9 ± 70.9	280.5 ± 101.4	266.0 ± 127.7	288.4 ± 43.6
2	296.1 ± 71.8	304.3 ± 89.3	265.4 ± 67.2	264.7 ± 20.7
3	264.5 ± 77.1	242.3 ± 93.0	196.7 ± 38.6	247.3 ± 38.8
4	153.9 ± 52.6	163.0 ± 62.8	144.5 ± 77.3	145.8 ± 80.4
5	234.3 ± 65.3	256.2 ± 101.7	199.3 ± 50.9	250.0 ± 82.0
6	256.4 ± 51.5	221.3 ± 76.8	229.1 ± 64.3	210.3 ± 74.6
Mean 0-14	246.3 ± 48.7	247.2 ± 61.0	222.3 ± 35.3	247.2 ± 61.0

[a] Data from Table 3, p. 28-31 of MRID 477751-01.

E. BLOOD ANALYSES:

1. **Hematology**: No statistically significant and/or biologically relevant differences were observed between hematology parameters measured on Day -1 and those of Day 1. No laboratory historical control data are given for these values, which are, however, reasonably consistent with those given in http://www.ahc.umn.edu/rar/refvalues.html as well as information given (p. 36 of MRID 47775102) in an adult dog study conducted by the same laboratory.

2. **Clinical Chemistry**: No statistically significant and/or biologically relevant differences were observed between clinical chemistry parameters measured on Day -1 and those of Day 1. No laboratory historical control data are given for these values, which are, however, reasonably consistent with those given in http://www.ahc.umn.edu/rar/refvalues.html as well as information given (p. 36 or MRID 47775102) in an adult dog study conducted by the same laboratory.

3. **Urinalysis:** No urinalysis measurements were made. Urinalysis is not required by the 870.7200 guidelines.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded (p. 6 of MRID 47775101) that the test substance X-6422-09 [containing 1.07% N-Octyl bicycloheptene dicarboximide; 0.529% Piperonyl butoxide; 0.307% Pyrethrins; 0.210% Bifethrin; 0.213% Permethrin; and 0.102% ETOC] "was safe to apply to puppies at 1x the label dose," X-6423-

09 [containing 3.19% N-Octyl bicycloheptene dicarboximide; 1.53% Piperonyl butoxide; 0.94% Pyrethrins; 0.604% Bifethrin; 0.614% Permethrin; and 0.308% ETOC] "was safe to apply to puppies at three times the label dose" and X-6414-08 [5.01% N-Octyl bicycloheptene dicarboximide; 2.45% Piperonyl butoxide; 1.64% Pyrethrins; 0.996% Bifethrin; 1.02% Permethrin; and 0.532% ETOC] "was safe to apply at five times the label dose." It would have been more appropriate to state that X-6422-09 was safe to apply at 1.9 g/kg, that X-6423-09 was safe to apply at 1.5 g/kg, and that XD-6414-08 was safe to apply at 1.6 g/kg.

B. REVIEWER COMMENTS:

Overall, there is no indication that exposure to the formulations (which included, for the 3x and 5x groups, exaggerated amounts of the active ingredients) resulted in any significant adverse toxicological effects. However, there are several deficiencies that should be corrected before this study can be classified as acceptable to support this product's use on 12-week old puppies. These are the following:

1) The composition of the placebo formulation (X-6415-08) should be provided, as well as information regarding the percentage(s) of inert(s) present in the other test formulations. In addition, a clarification is needed as on p. 6 of MRID 47775101 it is stated that: "On Day 0, Group I animals were treated with X-6415-08, a 5X dose of the placebo and served as controls." The mean dosage rate at which X-6415-08 was applied in Group I puppies was 1.7 g/kg, essentially the same dosage rate (1.9 g/kg) at which X-6422-09 was applied to Group II puppies, so it appears that the X-6415-08 was applied at a 1X application rate.

2) The dates of birth for the individual puppies should be reported to establish that they were in fact no more than 12 weeks old at treatment.

3) The product label should be revised to give dosage rates in terms of duration of spray (in seconds) based on a puppy's weight.

1. **DP BARCODE:** DP367106
2. **PC CODES:** 057001, 067501, 069001, 128825, 109701, 128722
3. **CURRENT DATE:** September 14, 2009
4. **TEST MATERIALS:** X-6422-09: N-Octyl bicycloheptene dicarboximide (1.07%); Piperonyl butoxide (0.529%); Pyrethrins (0.307%); Bifethrin (0.210%); Permethrin (0.213%); ETOC (0.102%) [used for 1x]. N-Octyl bicycloheptene dicarboximide (1.07%); Piperonyl butoxide (0.529%); Pyrethrins (0.307%); Bifethrin (0.210%); Permethrin (0.213%); ETOC (0.102%) [used for 1x]. X-6423-09: N-Octyl bicycloheptene dicarboximide (3.19%); Piperonyl butoxide (1.53%); Pyrethrins (0.940%); Bifethrin (0.604%); Permethrin (0.614%); ETOC (0.308%) [used as a 3x formulation]. X-6414-08: N-Octyl bicycloheptene dicarboximide (5.01%); Piperonyl butoxide (2.45%); Pyrethrins (1.64%); Bifethrin (0.996%); Permethrin (1.02%); ETOC (0.532%) [used as a 5x formulation].

Study/Species/Lab Study # / Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal safety /beagle puppies (~12 weeks old at study initiation)/Stillmeadow, Inc./Feb. 10, 2009	47775101	Groups of 6M & 6F 12-week old beagle puppies received topical (spray) applications of placebo, 1X, 3X and 5X formulations (3X & 5X formulations had exaggerated percentages of the actives) on Day 0, with subsequent 14 day observation. Mean doses ranged from 1.5-1.9 g/kg. No indication of any significant adverse toxicological effects. A number of deficiencies (including lack of dates of birth for puppies, historical control data for hematology and clinical chemistry data) were noted and should be addressed before the study can be classified as acceptable.	n/a	U (but study can be upgraded with additional information).

Core Grade Key: A = Acceptable, S = Supplementary, U = Unacceptable, W = Waived

EPA Reviewer: Byron Backus, Ph.D. **Signature:** Byron T. Backus
Technical Review Branch, Registration Division (7505P) **Date:** 9-14-2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion animal safety study- dogs; [OPPTS 870.7200]

PC CODES: 057001, 067501, 069001, 128825, 109701, 128722 **DP BARCODE:** 367106

TEST MATERIALS (PURITY): Placebo formulation (not characterized as to composition) used for Group I animals; -6422-09: X-6414-08: N-Octyl bicycloheptene dicarboximide (5.01%); Piperonyl butoxide (2.45%); Pyrethrins (1.64%); Bifethrin (0.996%); Permethrin (1.02%); ETOC (0.532%) used for Group II animals.

SYNONYM: X-6414-08 (CASS 5x Formula).

CITATION: Kuhn, J. (2009) X-6414-08: Companion Animal Safety Study in Dogs: Final Report. Project Number: 12315/08. Unpublished study prepared by Stillmeadow, Inc. 38 p. Unpublished. Feb. 10, 2009.

SPONSOR: McLaughlin Gormley King Company

EXECUTIVE SUMMARY: In a companion animal safety study (MRID 47775102), six adult (ages not specified) dogs/sex/group were topically sprayed on Days 0 and 7 with either a placebo (Group I) or a formulation (X-6414-08, containing 5.01% N-Octyl bicycloheptene dicarboximide; 2.45% Piperonyl butoxide; 1.64% Pyrethrins; 0.996% Bifethrin; 1.02% Permethrin; and 0.532% ETOC. Although the formulations did not include Nylar, the study in MRID 42178309 indicates a low mammalian toxicity for this active, and can be cited. The mean dosage rate for dogs sprayed with the X-6414-08 for both occasions was ~0.95 g/kg. Following treatment, individual dogs were observed at 1-hour intervals for 6 hours. They were observed (with a.m. and p.m. observations) for 21 days after the Day 0 application.

During the pretest period one Group II male favored one back leg on Days -16 through -12, and another Group II male had a growth/cyst between toes of the right foot, diagnosed as an interdigital abscess which was treated with full recovery by Day 1. One Group I male (4160-M) was not eating at the p.m. observations on Days -2 and -1; from the food consumption data this dog ate nothing on Days -11, -8, -4, -3, and -1, and consumed only 5 g of food on Day -2 and 6.2 g on Day 0. 4160-M was dehydrated on Day 1 and was treated [presumably intravenously] with 120 mL of sterile saline and was given antibiotics and fed Pedigree canned food on three occasions. This animal was also given an extra 25 g of regular food on at least four occasions (Days 14, 15, 16 and 20). From the food consumption data, several other dogs (Group II male 4161 on Days -11 and -12, Group II male 4163 on Day -11, Group I female 4180 on Day -6, Group II female 4177 on Day -5) had days when they did not consume any food.

From Day -20 to 0 Group I dogs went from a mean body weight of 8.3 to 7.2 kg, and Group II

dogs went from a mean body weight of 8.3 to 7.7 kg. No explanation is provided as to why these weight losses occurred.

During the immediate post-dose observations on Day 0 one Group II male had very slight lacrimation of the left eye at 6 hours and one Group II female had very slight lacrimation in one or both eyes at 1 hour through 6 hours; this same female also showed very slight lacrimation in both eyes at the pm observation on Day 0, the pm observation on Day 1 and the am observation on Day 2. Nothing was observed in any dog following treatment on Day 7.

One Group I male (4166-M) is reported as showing very slight salivation at the am observation on Day 1, one Group I female (4181-F) showed very slight lacrimation at the pm observation on Day 9 and a Group I female appeared thin on Days 20-21.

Four Group II dogs (4163-M, 4164-M, 4177-F and 4178-F) had low (<100 g) food consumption on Day 0, while only two Group I dogs (including 4160-M, which had not been eating well for several days) also had low food consumption. The mean food consumption for Group II females on Day 0 was 133.7 g, while for Group I females it was 225.1 g. Except for one male (4164-M) which consistently ate less than 200 g/day from Day 0 to 7, all Group II dogs consumed more than 200 g of food on Day 7. On Day 8 (the day following the second treatment), four (3 female, 1 male) Group II and two (both males) Group I dogs consumed less than 200 g. The food consumption data suggest (but do not conclusively demonstrate) a temporary decrease in appetite in some dogs, particularly females, following treatment with X-6414-08. However, this is not considered an indication of a serious adverse toxicological response.

Overall, there is no indication that exposure to X-6414-08 (which contained 5x amounts of the active ingredients) at a mean dose of 0.95 g/kg resulted in any significant adverse toxicological effects. However, there are several deficiencies that should be corrected before this study can be classified as acceptable to support this product's use on adult dogs. These are the following:

1) The composition of the placebo formulation (X-6415-08) should be provided, as well as information regarding the percentage(s) of inert(s) present in X-6414-08.

2) The approximate ages of the individual dogs should be reported.

3) There should be an explanation provided as to why a number of dogs had weight losses in the period from Day -2- to 0. During this period Group I dogs went from a mean body weight of 8.3 to 7.2 kg, and Group II dogs went from a mean body weight of 8.3 to 7.7 kg. An explanation should also be provided as to why Group I male 4160-M was included in this study, as this animal was eating very little in the period from Day -5 through Day 0 (according to the food consumption data this dog ate a total of 34.9 grams of food during this period), and was obviously not in good physical condition as it was dehydrated on Day -1.

4) Information should be given as to whether the dogs were fed before or after treatment on Days 0 and 7.

5) The product label should be revised to give dosage rates in terms of duration of spray (in seconds) based on a dog's weight.

This companion animal safety study (OPPTS 870.7200) in adult dogs is currently classified as unacceptable. It can be upgraded to acceptable provided the deficiencies indicated above are satisfied.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided, although on p. 3 of MRID 47775102 it is stated: "It is not known if the provided analysis for characterization and stability was conducted according to Good Laboratory Practice Standards."

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** X-6414-08 (CASS 5x Formula)

Description:	Milky white liquid
Batch #:	
Purity:	N-Octyl bicycloheptene dicarboximide (5.01%); Piperonyl butoxide (2.45%); Pyrethrins (1.64%); Bifethrin (0.996%); Permethrin (1.02%); ETOC (0.532%) [used for 5x].
Compound Stability:	"September 2010 per provided information."
CAS #:	Not provided

2. **Vehicle control:** A placebo control (X-6415-08) of the formulation without active ingredients was used. The expiration date was September 2010 "per provided information." No information is provided as to its composition.

3. **Test animals:**

Species:	Dog
Strain:	Beagle
Age/weight at study initiation:	Ages not reported. Day -20 weights ranged from 6.7 – 10.2 kg for males and 7.0 to 9.2 kg for females. Day 0 weights ranged from 5.4 – 10.9 kg for males and 5.4 – 8.3 kg for females.
Source:	STILLMEADOW Dog Colony, Sugar Land, TX
Housing:	Individual cages measuring 3' x 4', with wire bottoms.
Diet:	Canine High Density Diet #5L18 (PMI Nutrition International, Inc.), fed once a day "appropriate amount to meet nutritional requirements." From the maximum food consumption values, each dog was offered 250 grams of food from Day -21 to Day 6; some dogs are reported as consuming more than 250 g on Day 7 (up to 290.5 g in one case); then on Day 8 the maximum amount any dog consumed was 250 g; on Days 9-12 some consumed more than 250 g; then for Days 13-15 the maximum amount consumed was 250 g.
Water:	Municipal supply, *ad libitum*
Environmental conditions:	**Temperature:** 15-25^0 C **Humidity:** 42-98% **Air changes:** 10-12 exchanges/hour **Photoperiod:** 12 hrs light/12 hrs dark
Acclimation period:	~ 3 weeks

B. STUDY DESIGN:

1. **In life dates:** From p. 7 of MRID 47775102: "The study was initiated on 17 Sep 08, and the laboratory portion of the study was conducted from 23 Sep. 08 to 14 Oct 08."

2. **Animal assignment:** From p. 9 of MRID 47775102: "Using a computer-generated program, animals were randomly assigned to two groups, Group I and II with six males and six females in each group in an effort to equalize the mean body weight and sex."

TABLE 1. Study design[a]

Test Group	Number of dogs	Dosages (with mean±S.D.) Applied (in g)	Day 0 & 7 Body wts. in kg (mean)	Day 0 & 7 Dosages (mean) in g/kg
I (Placebo)	12 (6 M and 6 F)	Day 0: M: 5.4-7.7 (6.57±0.83) F: 4.9-6.8 (5.90±0.62) Day 7: M: 6.2-8.5 (6.95±0.91) F: 5.8-7.9 (6.90±0.93)	0: M: 5.4-8.5 (7.25) F: 5.4-8.3 (7.17) 7: M: 6.7-10.2 (8.37) F: 6.6-9.2 (7.60)	0: M: 0.68-1.24 (0.93) F: 0.75-0.91 (0.84) 7: M: 0.70-0.96 (0.84) F: 0.78-1.11 (0.91)
II (5x)	12 (6 M and 6 F)	Day 0: M: 6.9-8.1 (7.57±0.44) F: 6.3-7.7 (6.67±0.55) Day 7: M:6.7-9.4 (7.95±0.92) F: 6.2-8.5 (7.42±0.87)	0: M: 6.4-10.0 (8.20) F: 6.6-7.8 (7.23) 7: M: 6.4-10.6 (8.65) F: 6.9-8.1 (7.47)	0: M: 0.77-1.22 (0.95) F: 0.82-1.10 (0.93) 7: M: 0.74-1.29 (0.94) F: 0.87-1.10 (0.99)

[a] Calculated from data from p. 13 of MRID 47775102

3. **Dose selection rationale:** From p. 7 of MRID 47775101: "This study was conducted to evaluate the potential toxicity of the test substance when applied topically to adult dogs at five times the label dose." According to the 870.7200 Companion Animal Safety Guidelines, testing of formulations specifically prepared for a study of this type that contain higher concentrations (3x and 5x) of the active ingredient[s] is not only acceptable, but "preferred" and "If a test at one dose level of at least 5X the recommended dose…produces

no evidence of treatment-related toxicity, a full study using a minimum of three dose levels may not be necessary."

4. **Preparation and treatment**: On Days 0 and 7 the test and control formulations were administered topically. The containers were held upright 6-10 inches from the animal and were moved as the spray was discharged. The spray was "lightly" applied to the entire body with an even coverage until the tips of the hair were moist. The formulation was rubbed into the coat by ruffling the hair to reach the skin. Containers were weighed before and after dosing to determine the amount applied on each dog. It is stated (p. 9 of MRID 47775102) that Group I dogs were treated with a 5X dose of the placebo, X-6415-08; however, from information on p. 13 Group I dogs were treated with from 0.84-1.24 g/kg, comparable to the dosage range of 0.77-1.29 g/kg Group II dogs received for the 5X formulation.

5. **Statistics**: Individual values and group mean values (with standard deviations) by sex are presented for a number of parameters (food consumption, body weights, serum chemistry, and hematology). However, there is no indication within the report (except possibly the statement on p. 12 of MRID 47775102 that there were no significant changes from Day -1 to Day 1 for hematology and serum chemistry results and therefore no blood was drawn on Day 8) that these values were statistically analyzed.

C. **METHODS**:

1. **Observations and pre-exposure treatments:** All dogs were observed twice daily from Day -21 through Day 21; in addition, on study days 0 and 7 they were also observed hourly for 6 consecutive hours after treatment. Only abnormalities were recorded.

2. **Body weight**: Body weights were recorded on Days -20, -1, 7, 14 and 21.

3. **Food consumption**: The dogs were fed once a day. Individual daily food consumption values are provided from Day -21 through Day 21. From the maximum food consumption values, it appears that the dogs were offered 250 g/dog/day from Day -21 through Day 6, that at least some dogs were offered more on Day 7 (maximum individual consumption value for that date: 290.5 g), but were then offered 250 g/dog/day on Day 8. Some individual daily food consumption values were >250 g on Days 10 through 12, then the maximum was 250 g for the remainder of the study except for one Group I dog (4160-M) which was offered 275 g on at least some days (this dog is reported on p. 11 as not having eaten on Days -2 and -1 and as being dehydrated on Day 0).

4. **Hematology & Clinical Chemistry**: Blood was collected from the jugular vein for hematology and clinical chemistry assessments for all dogs on Days -1 and 1. The CHECKED (X) parameters were examined.

a. **Hematology**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count		Reticulocyte count
	Blood clotting measurements*		Large unstained cells (LUC)
	(Thromboplastin time)		Fibrinogen
X	(Activated Partial Thromboplastin Time)	X	Heinz Bodies
X	(Prothrombin time)		

* Recommended for companion animals safety evaluation based on OPPTS 870.7200

b. **Clinical Chemistry**

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Sodium/Potassium ratio	X	Urea nitrogen*
X	Phosphorus *		Total Cholesterol
X	Potassium* (K)	X	Globulins*
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, eg.,)	X	Total bilirubin *
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Albumin/Globulin ratio
	Lactic acid dehydrogenase (LDH)	X	Direct bilirubin*
X	Alanine aminotransferase (ALT/also SGPT)*		Indirect bilirubin
X	Aspartate aminotransferase (AST/also SGOT)*		BUN/Creatinine ratio
	Gamma glutamyl transferase (GGT)		TCO_2 Bicarbonate
	Glutamate dehydrogenase		Amylase
	Sorbitol dehydrogenase		Lipase

* Recommended for a companion animal safety evaluation based on OPPTS 870.7200

5. **Urinalysis:** No urinalysis was performed. Urinalysis is not specified in the 870.7200 Guidelines.

6. **Sacrifice and Pathology**: None of the dogs died during the course of the study, and a terminal sacrifice is not required for this type of study.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** During the pretest period one Group II male (4166-M) favored one back leg (Days -16 through -12), and another Group II male (4171-M) had a growth/cyst between toes of the right foot (Days -12 through -6); this was diagnosed as an interdigital abscess and was treated, with full recovery by Day 1. One Group I male (#4160-M) was not eating at the pm observations on Days -2 and -1; according to the food consumption data

(pages 23-25) ate nothing on Days -11, -8, -4, -3, and -1, and consumed only 5 g of food on Day -2 and 6.2 g on Day 0. 4160-M was dehydrated on Day 1 and was treated [presumably intravenously] with 120 mL of sterile saline, given antibiotics and was fed Pedigree canned food on three occasions. This animal was also given an extra 25 g of regular food on at least four occasions (Days 14, 15, 16 and 20). From the food consumption data (Table 4 of MRID 47775102), several other dogs (Group II male 4161 on Days -11 and -12, Group II male 4163 on Day -11, Group I female 4180 on Day -6, Group II female 4177 on Day -5) had days when they did not consume any food.

From Day -20 to 0 Group I dogs went from a mean body weight of 8.3 to 7.2 kg, and Group II dogs went from a mean body weight of 8.3 to 7.7 kg. No explanation is provided as to why these weight losses occurred.

During the immediate post-dose observations one Group II male had very slight lacrimation of the left eye at 6 hours and one Group II female had very slight lacrimation in one or both eyes at 1 hour through 6 hours; this same female also showed very slight lacrimation in both eyes at the pm observation on Day 0, the pm observation on Day 1 and the am observation on Day 2. Nothing was observed in any dog following treatment on Day 7.

One Group I male (4166-M) is reported as showing very slight salivation at the am observation on Day 1, one Group I female (4181-F) showed very slight lacrimation at the pm observation on Day 9 and a Group I female appeared thin on Days 20-21.

2. **Cosmetic effects**: No cosmetic effects were observed.

3. **Mortality**: There was no mortality.

B. **BODY WEIGHT AND WEIGHT GAIN**: Select mean body weight data are presented in Table 2. Group I males and females gained more body weight than Group II males and females from Day 0 to 21, but body weight changes between Day -21 and 20 were essentially the same.

TABLE 2. Mean body weight ± S.D. (kg) in dogs treated with X-6414-08[a]		
Day	**Group I. Placebo**	**Group II. 5x**
Males		
-20	8.58 ± 1.29	8.68 ± 1.37
0	7.25 ± 1.33	8.20 ± 1.31
7	8.37 ± 1.33	8.65 ± 1.59
14	8.40 ± 1.34	8.55 ± 1.59
21	8.62 ± 1.34	8.68 ± 1.44
Change Day -20 to Day 21	0.03 ± 0.36	0.00 ± 0.38
Change Day -20 to Day 0	-1.33 ± 0.20	-0.48 ± 0.58
Change Day 0 to Day 21	1.37 ± 0.42	0.48 ± 0.68
Females		
-20	7.92 ± 0.74	7.95 ± 0.67
0	7.17 ± 1.00	7.23 ± 0.48
7	7.60 ± 0.94	7.47 ± 0.51
14	7.67 ± 0.91	7.40 ± 0.56
21	7.78 ± 1.08	7.45 ± 0.48
Change Day -20 to Day 21	-0.13 ± 0.65	-0.50 ± 0.36
Change Day -20 to Day 0	-0.75 ± 0.78	-0.72 ± 0.32
Change Day 0 to Day 21	0.62 ± 0.67	0.22 ± 0.10

[a]Values calculated from Table I, page 13 in MRID 47775102.

C. **FOOD CONSUMPTION:**

Select mean food consumption data are presented in Table 3. Four Group II dogs (4163-M, 4164-M, 4177-F and 4178-F) had low (<100 g) food consumption on Day 0, while only two Group I dogs (this includes 4160-M, which had not been eating well for several days) were in this category. However, except for one male (4164-M) which consistently ate less than 200 g/day from Day 0 to 7, all Group II dogs consumed more than 200 g of their food on Day 7. On Day 8, four (3 female, 1 male) Group II and two (both males) Group I dogs consumed less than 200 g. The food consumption data suggest (but do not conclusively demonstrate) a temporary decrease in appetite in some dogs, particularly females, following treatment with X-6414-08. However, this is not considered an indication of a serious adverse toxicological response.

TABLE 3. Mean daily food consumption ± S.D. (g) in dogs treated with X-6414-08 and their controls[a]		
Day	**Group I (placebo)**	**Group II (5x)**
	Males	
-3	170.5 ± 95.6	144.8 ± 68.9
-2	175.8 ± 90.3	195.2 ± 41.6
-1	197.0 ± 98.1	174.7 ± 47.4
0	171.6 ± 101.9	149.2 ± 76.0
1	156.9 ± 90.3	188.6 ± 61.6
2	183.3 ± 86.0	205.9 ± 43.2
3	234.0 ± 21.6	219.7 ± 29.7
4	235.5 ± 24.7	230.9 ± 24.4
5	235.0 ± 31.0	235.2 ± 25.5
6	243.6 ± 15.7	240.9 ± 22.4
7	251.0 ± 25.4	224.3 ± 44.6
8	199.2 ± 70.8	211.6 ± 46.4
9	230.8 ± 38.1	233.0 ± 27.9
10	244.9 ± 47.9	193.2 ± 48.4
	Females	
-3	203.6 ± 51.4	153.2 ± 60.6
-2	226.2 ± 21.4	149.0 ± 38.7
-1	209.5 ± 33.2	177.9 ± 51.5
0	225.1 ± 61.1	133.7 ± 90.5
1	199.1 ± 87.8	165.8 ± 54.7
2	245.4 ± 11.3	204.7 ± 37.4
3	236.5 ± 17.9	152.3 ± 74.1
4	245.6 ± 10.8	188.1 ± 43.3
5	250.0 ± 0.0	223.5 ± 45.5
6	244.2 ± 9.4	238.7 ± 17.6
7	251.6 ± 19.8	245.2 ± 23.8
8	250.0 ± 0.0	181.4 ± 54.8
9	244.4 ± 13.9	205.7 ± 40.0
10	253.8 ± 17.3	225.9 ± 27.6

[a] Data from Table 3, p. 28-31 of MRID 477751-01.

E. BLOOD ANALYSES:

1. **Hematology**: No statistically significant and/or biologically relevant differences were observed between hematology parameters measured on Day -1 and those of Day 1. The report includes (p. 36 of MRID 47775102) reference ranges for these parameters. Most of the individual measurements were within these reference ranges.

2. **Clinical Chemistry**: No statistically significant and/or biologically relevant differences were observed between clinical chemistry parameters measured on Day -1 and those of Day 1. The report includes (p. 36 of MRID 47775102) reference ranges for these parameters. Most of the individual measurements were within these reference ranges.

3. **Urinalysis:** No urinalysis measurements were made. Urinalysis is not required by the 870.7200 guidelines.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded (p. 6 of MRID 47775102) that the test substance X-6414-08 [5.01% N-Octyl bicycloheptene dicarboximide; 2.45% Piperonyl butoxide; 1.64% Pyrethrins; 0.996% Bifethrin; 1.02% Permethrin; and 0.532% ETOC] "was safe to apply to adult dogs at five times the label dose." It would have been more appropriate to state that X-6414-08 was safe to apply to adult dogs at 0.95 g/kg.

B. REVIEWER COMMENTS:

Overall, there is no indication that exposure to X-6414-08 (which contained 5x amounts of the active ingredients) resulted in any significant adverse toxicological effects. It is possible that there were some minor effects (slight lacrimation, as seen in Group II female 4181-F at 1-6 hours after dosing on Day 0, and at the pm observation on Day 1 and the am observation on Day 2; and reduced food consumption for some Group II females on Day 8). However, there are several deficiencies that should be corrected before this study can be classified as acceptable to support this product's use on adult dogs. These are the following:

1) The composition of the placebo formulation (X-6415-08) should be provided, as well as information regarding the percentage(s) of inert(s) present in X-6414-08.

2) The approximate ages for the individual dogs should be reported.

3) Information should be given as to whether the dogs were fed before or after treatment on Days 0 and 7.

4) There should be an explanation provided as to why a number of dogs had weight losses in the period from Day -20 to 0. During this period Group I dogs went from a mean body weight of 8.3 kg to 7.2 kg, and Group II dogs went from a mean body weight of 8.3 to 7.7 kg. An explanation should also be provided as to why Group I male 4160-M was included in this study, as this animal was eating very little in the period from Day -5 through Day 0 (according to the food consumption data this dog ate a total of 34.9 grams of food during this period), and was obviously not in good physical condition as it was dehydrated on Day -1.

5) The product label should be revised to give dosage rates in terms of duration of spray (in seconds) based on a dog's weight.

1. **DP BARCODE:** DP367106
2. **PC CODES:** 057001, 067501, 069001, 128825, 109701, 128722
3. **CURRENT DATE:** September 14, 2009
4. **TEST MATERIALS:** X-6414-08: N-Octyl bicycloheptene dicarboximide (5.01%); Piperonyl butoxide (2.45%); Pyrethrins (1.64%); Bifethrin (0.996%); Permethrin (1.02%); ETOC (0.532%) [used as a 5x formulation].

Study/Species/Lab Study # / Date	MRID	Results	Tox. Cat.	Core Grade
Companion animal safety /adult dogs (ages not specified)/Stillmeadow, Inc./Feb. 10, 2009	47775102	Groups of 6M & 6F adult dogs (ages not specified) received topical (spray) applications of placebo or 5X formulations (~0.95 g/kg) on Days 0 and 7, with observations to Day 21. Group I male 4160-M ate nothing Days -4, -3, and -1, ate only 5 g of food on Day 2 and 6.2 g on Day 0, and was dehydrated on Day 1 and treated with sterile saline. From Day -20 to 0 Group I dogs went from a mean wt of 8.3 to 7.2 kg, and Group II dogs went from a mean wt of 8.3 to 7.7 kg. No explanation is provided as to why these wt losses occurred. On Day 0 one Group II female had very slight lacrimation in one or both eyes and this continued to am of Day 2. Food consumption data for Days 0 & 8 suggest a temporary decrease in appetite for some Group II dogs, particularly females. No indication of any significant adverse toxicological effects, but study has a number of deficiencies that must be addressed.	n/a	U (but study can be upgraded with additional information).

Core Grade Key: A = Acceptable, S = Supplementary, U = Unacceptable, W = Waived